Environmental and Experimental Botany 67 (2009) 284–292

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Microsite conditions influence nutritive value characteristics of a tall fescue cultivar devoid of, or infected with a native, or a novel non-ergogenic endophyte

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ARTICLE INFO

Article history:
Received 11 February 2009
Received in revised form 24 June 2009
Accepted 16 July 2009

Keywords:
Crude protein
Ergoalkaloids
Evapotranspiration
Leaf dry matter content
Non-structural carbohydrate
Phenolics
Loline alkaloids
Radiation productivity
Shade

ABSTRACT

Tall fescue [*Lolium arundinaceum*, Schreb., S.J. Darbysh.] productivity and persistence often benefits from association with *Neotyphodium coenophialum* [Morgan-Jones and Gams], Glenn, Bacon, and Hanlin) endophyte. The influence of novel, non-ergogenic endophytes on nutritive value is unclear, especially when simultaneous stresses (*e.g.*, defoliation and shading) are imposed on the association. We conducted a field experiment using Jesup tall fescue that had either a native or novel non-ergogenic fungal endophyte (AR542; referred to as MaxQ™), or that was endophyte free. Dry matter production and nutritive value including crude protein (CP), non-structural carbohydrates (TNC), ergo- and loline alkaloids, and phenolics were determined for plants stockpiled or clipped repeatedly in sites differing in the amount of light. Productivity varied less among sites when plants were infected with a native endophyte compared to novel or no endophyte. The trend suggests that native endophyte contributed to resilience of the host in this experiment. Leaf dry matter content was affected by host–endophyte association interacting with light availability suggesting differences in leaf composition could occur. Herbage CP increased, whereas TNC decreased with increasing shade. The concentration of loline alkaloids, irrespective of host–endophyte association, tended to increase in leaves with decreasing light availability and could be related to the relatively greater N concentrations in shade-grown leaves. Phenolics decreased in leaves, but increased in stembases as light availability decreased. The combination of increased loline alkaloids in leaves and phenolics in stembases, suggests that shade-grown tall fescue might have some competitive advantage based on the known anti-herbivory attributes of loline alkaloids and phenolic compounds.

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1. Introduction

Environmental and edaphic conditions help define the range of successful adaptation and the production limits of many forage grasses. The plants often are subjected to multiple simultaneous stresses associated with site features and management practices. Ecophysiological experiments with plants subjected to episodic defoliation, simulating grazed plants, are not common because of the disruption of light acquisition and the confounding influences of management. Interactions complicate explanations of long-term responses, such as total production in a growing season or persistence, which usually are based on instantaneous measurements made at fixed points during or at the end of a growth interval. As an example, topography and the mosaic of open pasture and woodland found on many small-scale farming operations affects the amount of light reaching the forage canopy. We found that sites at the boundary of woodlands and pasture seemed to support better forage production and nutritive value than the extremes of open or densely shade sites (Belesky et al., 2006; Neel et al., 2008).

Grasses growing in shaded sites were often endophyte-infected, which suggests some beneficial aspect of fitness when infection occurs (Clay and Leuchtmann, 1989). Tall fescue [*Lolium arundinaceum*, Schreb., S.J. Darbysh.] is an important agronomic species that often is infected by a mutualistic endophytic fungal symbiont (*Neotyphodium coenophialum* [Morgan-Jones et Gams] Glenn, Bacon, and Hanlin). This association seems to contribute to adaptability to the dynamic growing conditions occurring in agroecosystems in humid temperate regions (Malinowski and Belesky, 2006). For many years, endophyte-infected forage grasses were considered detrimental because of deleterious impacts on livestock performance and health. Currently, endophyte-infected grasses are considered beneficial in pastoral systems (Bouton et al., 2002; Hill et al., 2002). This change in perception occurred in part because novel endophytes with desirable characteristics were developed, which could then be inserted into host grasses. Among these are non-ergogenic endophytes that do not impair the performance and health of grazing livestock (Parish et al., 2003). However, not much is known about how novel endophyte–host associations

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0098-8472/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.envexpbot.2009.07.008

partition photosynthate or whether novel endophytes, interacting with sites, influence nutritive value. Recently, Rasmussen et al. (2007, 2008) noted that novel endophytes interacted with primary metabolism (non-structural carbohydrate and soluble protein) of the host plant and that this interaction could influence the functioning of host–endophyte associations in grassland ecosystems.

Among the earliest discoveries concerning endophyte in tall fescue was the apparent efficient conversion of N to growth (Arachevaleta et al., 1989). Shade-grown, cool-temperate origin grasses such as orchardgrass (*Dactylis glomerata* L.) increase allocation of N to leaves (Belesky et al., 2006), in part, to optimize light utilization. The N accumulation occurs along with relatively low amounts of current or stored photosynthate in the form of non-structural carbohydrate. Unassimilated N along with depressed levels of total non-structural carbohydrates (TNC) in shade-grown herbage (Belesky et al., 2006) could compromise nutritive value (Neel et al., 2008) and influence livestock grazing behavior (Mayland et al., 2000). Could endophyte help overcome the accumulation of excessive amounts of N in tall fescue growing in partially shaded sites? Rasmussen et al. (2007) provided some clues regarding the influence of host–endophyte associations differing in TNC accumulation on primary metabolism of the host plant. In their work, high N supply significantly reduced concentrations of endophyte and alkaloids in perennial ryegrass (*L. perenne*, L.). Their work also suggests that the fungal genome is likely to have a much greater influence than first thought on host metabolism (Rasmussen et al., 2008).

Photosynthate in excess of that needed for growth accumulates as TNC in the stembase of cool-season grasses and provides energy for regrowth after defoliation; however, defoliation frequency (intervals between events) or intensity (plant residual height after an event) can impair TNC replenishment. Plant management practices that retain stembase after defoliation seem to benefit leaf regeneration and plant persistence (Fulkerson and Donaghy, 2001). Again, Rasmussen et al. (2007) noted that high non-structural carbohydrate concentrations suppressed endophyte and alkaloid in host leaves.

Deficits in photosynthate that occur in grasses growing in partially shaded sites might influence ergoalkaloid or other secondary metabolite concentrations. Ergoalkaloid concentrations were depressed in tall fescue subjected to repeated defoliation, which very likely depleted readily available energy (Belesky and Hill, 1997). The concentration of phenolic compounds in endophyte-infected tall fescue increased with nutrient stresses (Malinowski et al., 1998). Phenolics could influence plant response to shade (Close and McArthur, 2002), impact plant persistence through resistance to herbivory and disease (Mauch-Mani and Metraux, 1998), and influence herbage nutritive value (Burns, 1966). There is some evidence that phenolics operate as photosystem protectants, and that production is stimulated by high red:far-red quotients typical of open sites (Feldhake et al., 2005). Our objective was to determine if site, characterized by light availability, and defoliation intensity influenced herbage nutritive value including loline and ergoalkaloids, and phenolics as a function of tall fescue–endophyte association. We anticipate that findings will lead to forage management strategies that optimize leaf DM production and nutritive value for tall fescue grown in temperate region silvopasture or partially shaded environments.

2. Materials and methods

2.1. Plant culture

Details of experiment design, plant culture and sampling protocol appear in Belesky et al. (2008). Briefly, seed of Jesup infected with a wild-type endophyte (J+), Jesup devoid of endophyte (J−), and Jesup containing the novel endophyte AR542 and hence referred to as MaxQ™,[1] was sown in 2.5 L plastic pots containing a mixture of 2 parts soil (Gilpin, fine-loamy, mixed mesic, Typic Hapludult) and 1 part sand (mixture pH ~ 7.0). Plants were grown for six weeks in a controlled environment room, with a 14 h photoperiod, 24/18 °C light/dark temperature and 55% relative humidity. Plants were watered as needed. Plants were maintained out-of-doors for 18 d in a non-shaded area prior to placement at sites in early May. Sites (81°7′W; 37°45′N; 755 m above sea level) included an open (OP; full sunlight) unobstructed, and two edge zones representing a north (NE; about 40% light attenuation relative to full sunlight) and south (SE; about 80% light attenuation relative to full sunlight) edge of a forest opening parallel to the sun path. Values for evapotranspiration (ET_o) representing potential evaporative water loss were calculated according to the Penman–Monteith equation (Monteith and Unsworth, 1990) based on air temperature, solar radiation, wind speed, relative humidity, longitude, latitude and elevation above sea level (Belesky, 2005).

A split application of $Ca(NO_3)_2$ and KH_2PO_4 provided a total of 120, 160, and 200 kg ha^{-1} N, P, and K, with half of the season total applied when plants were placed at each site, and half applied at mid season. Microclimate data were collected continuously throughout the growing season using automated weather stations at each site (Belesky et al., 2008).

2.2. Sample collection and analysis

Baseline data were collected from 18 replicates (9 replicates of the 5-cm residual plant height and 9 replicates of the 10-cm residual plant height), immediately prior to placement, at which time all plants were clipped to their respective 5-cm or 10-cm residual plant height. Three replicates of each of the three tall fescue associations were collected and a destructive sampling made each time mean plant height reached 20 cm at each site. Mass of leaf (>5-cm or 10-cm above soil surface) and stembase (soil surface to 5-cm or 10-cm) was determined. Leaf dry matter content (LDMC) was computed as: 100 − (wet weight − dry weight/wet weight × 100).

Plants were clipped to the respective residual height and allowed to regrow to 20 cm. Plants clipped to the 10 cm residual height were clipped six times, regardless of site, whereas plants clipped to 5 cm were clipped four times at OP, three times at NE, and four times at the SE sites with the exception of J− which was clipped only three times. Three replicates of each association were maintained as undisturbed canopies (stockpiled) at each site until the end of the growing season. The undisturbed plants were removed in late autumn and partitioned into leaf, stem, root, and senesced tissue. Tissues were lyophilized, ground in an Udy cyclone mill to pass a 1 mm sieve, and weighed to determine dry mass. Lyophilized, and ground plant tissue was used for all chemical component analyses.

Crude extracts of leaf and stembase tissues were made according to Terrill et al. (1992) and Waterman and Mole (1994), and relative total phenolic concentrations determined by the Price and Butler method (Waterman and Mole, 1994).

Loline alkaloids were extracted from the dried and pulverized plant material with ethanol:methylene chloride (4:1, v/v) containing internal standard quinoline and sodium bicarbonate (Blankenship et al., 2001). Individual alkaloids were resolved and quantified by gas chromatography with flame ionization detection. A 15 m × 0.53 mm DB5 column was maintained at an initial oven temperature of 70 °C, increased to 160 °C at 45 °C min^{-1}, held for 5 min and increased to 290 °C at 45 min^{-1} and held for 7 min. Detec-

[1] Trade names are used for the convenience of the reader and do not imply endorsement by USDA over comparable products and services.

Table 1
Analysis of variance for radiation productivity (RP) leaf dry matter content (LDMC), leaf or stembase total non-structural carbohydrate (TNC), leaf or stembase crude protein (CP), and total digestible nutrients (TDN) to protein quotient (TDN:CP) of leaves of Jesup+, Jesup−, and MaxQ™ tall fescue associations (FA) stockpiled, or clipped to a 5-cm or 10-cm residue height (RH) as a function of site (S) including OP, full sunlight; NE; about 60% of full sunlight; SE, about 20% of full sunlight, for data representing repeated measurements of three replicates during the growing season.

Clipped	RP (258)[a]		LDMC (258)		CP (516)				TNC (516)				TDN:CP (516)	
	F	*P*>*F*	*F*	*P*>*F*	Leaf		Stembase		Leaf		Stembase		*F*	*P*>*F*
					F	*P*>*F*	*F*	*P*>*F*	*F*	*P*>*F*	*F*	*P*>*F*		
FA	5.42	**	1.58	ns	0.04	ns	0.02	ns	0.70	ns	0.21	ns	1.17	ns
S	104.39	***	37.61	***	18.80	***	39.55	***	0.87	ns	1.43	ns	7.64	*
RH	9.95	**	6.98	**	45.59	***	44.67	***	0.20	ns	0.13	ns	11.82	***
FA*S	2.00	ns	2.28	ns	0.08	ns	0.48	ns	2.27	*	1.36	ns	2.56	*
FA*RH	1.66	ns	0.10	ns	0.61	ns	0.26	ns	0.47	ns	2.93	ns	0.04	ns
S*RH	4.38	*	0.67	ns	9.70	***	2.15	ns	6.40	**	11.78	***	1.09	ns
FA*S*RH	2.87	*	0.23	ns	0.54	ns	0.76	ns	1.51	ns	1.80	ns	0.74	ns
Stockpiled[b]														
FA	4.65	*	0.35	ns	0.06	ns	1.37	ns	0.09	ns	0.34	ns	0.14	ns
S	250.97	***	5.91	*	6.36	**	2.30	ns	17.10	***	4.18	*	9.99	**
FA*S	4.74	**	0.06	ns	0.14	ns	0.29	ns	0.15	ns	0.40	ns	0.10	ns

Representing repeated measurements of three replicates during the growing season. ns, not significant.
[a] Degrees of freedom.
[b] 27 degrees of freedom for stockpiled data analyses
* $P<0.05$.
** $P<0.01$.
*** $P<0.001$.

tion limit was $10\,\mu g\,g^{-1} \pm 35\,\mu g\,g^{-1}$ which can vary with column and gas chromatograph conditions.

Ergovaline and ergovalinine were determined using HPLC (Yates and Powell, 1988) equipped with a fluorescence detector with excitation at 310 nm and measurement at 420 nm. Separation was made on an Alltech Alltima C18 150 mm × 4.6 mm column with 3 μm particle size. Elution was made with (a) 75 mM ammonium acetate in water:acetonitrile (3:1, v/v), and (b) acetonitrile. Elution gradient was 95:5 (a:b) for 1 min; linear change to 60:40 (a:b) during next 15 min and maintained for 5 min; changed to 0:100 (a:b) in 1.5 min and maintained for 5 min; changed to 100:0 (a:b) in 1 min and maintained for 6 min before returning to the initial 95:5 (a:b) eluant ratio. Detection limit was $0.02\,\mu g\,g^{-1} \pm 0.05\,\mu g\,g^{-1}$ that varies with column and changes slightly over time. Reported values in the text represent the sum of ergovaline and ergovalinine.

2.3. Computation of radiation productivity, and estimates of nutritive components and total digestible nutrients of herbage

Radiation productivity (RP) represents dry matter productivity ($kg\,DM\,ha^{-1}\,d^{-1}$) expressed as a function of mean solar radiation ($MJ\,m^{-2}\,d^{-1}$) measured above the canopy near each site for a given growth interval. RP is not based on intercepted solar radiation, but rather mean solar radiation during the entire growth interval at a given site. Values may be used for comparative purposes and are not meant as a measure of the photosynthetic efficiency for a particular canopy.

Total non-structural carbohydrates were determined by an automated hydrolysis method (Denison et al., 1990). Nitrate content was determined by ion chromatography (Dionex DX 500 I.C.). Nitrogen expressed as crude protein ($N\,g\,kg^{-1}$ DM × 6.25) was determined by combustion of dry plant tissue using a Carlo Erba EA 1108 CHNSO analyzer (Fisons Instruments, Beverly, MA, USA).

Dried tissue samples were analyzed for *in vitro* organic matter disappearance (IVOMD) using rumen fluid obtained from rumen-cannulated steers (*Bos taurus*) offered orchardgrass–alfalfa (*Medicago sativa* L.) hay (Moore, 1970). Computations for nutritive value included, crude protein (CP) $g\,100\,g^{-1} = (\text{total N}\,g\,100\,g^{-1} \times 6.25)$ and metabolizable energy of feed (ME) as $ME\,(MJ\,kg^{-1}\,DM) = 0.0157$ (IVOMD) (AFRC, 1993). Total digestible nutrients (TDN) were calculated from ME data (NRC, 1996).

2.4. Statistical analysis

Data collected represent ecophysiological (RP, LDMC) and nutritive value (TNC, CP, TDN:CP, loline and ergoalkaloids, and phenolics) responses to site and treatments. Microclimate data were subjected to SAS principal component analysis procedures. Radiation productivity, TNC, crude CP, TDN:CP, and secondary metabolites (ergoalkaloids and loline alkaloids, and total phenolics) data were analyzed using mixed model procedures (SAS Inst., Cary, NC, USA). Replication and interactions with replication were considered random. Fescue association (J+, J−, MaxQ™), residual plant height (5 cm or 10 cm), and site (open, OP; north edge, NE; south edge, SE) modeled as fixed effects, and were analyzed as repeated measures using SUBJECT and GROUP options of SAS. Radiation productivity and LDMC data were subjected to regression analysis to determine goodness-of-fit based on host–endophyte association, residue height and seasonal distribution.

3. Results and discussion

3.1. Radiation productivity and leaf dry matter content

Sites ($F_{\text{d.f. }528}$ 267704; $P \leq 0.001$), residual plant height ($F_{\text{d.f. }528}$ 30323; $P \leq 0.001$), and the interaction of residual height and site ($F_{\text{d.f. }528}$ 463; $P \leq 0.001$) influenced ET_o. The ET_o values integrate microclimate features including irradiance with plant physiological response to canopy management. The duration of growth intervals differed with management and site (Belesky et al., 2008) and contributed to differences in ET_o arising from differing amounts of data used to compute ET_o. Site and residual clipping height also interacted to influence ET_o because the duration of time required to achieve the target canopy height of 20 cm differed. Plants clipped to 10 cm had to extend 10 cm of leaf, whereas plants clipped to 5 cm had to extend 15 cm of leaf to reach the target 20 cm canopy height. When compared to the 5-cm residual clipping height plants, those clipped to the 10 cm residual clipping height had relatively older tissues because a greater proportion of the total above-ground mass was retained in the residual herbage. Older tissues tend to have relatively lower stomatal conductance. Principal component analysis showed that ET_o associated with OP, NE and SE sites (designations used hereafter to describe sites) accounted for 95% of the variabil-

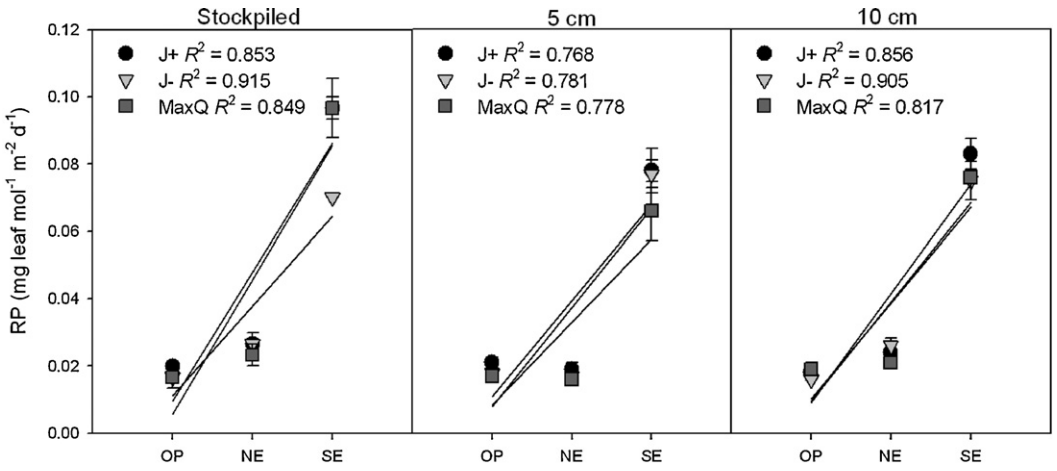

Fig. 1. Radiation productivity (mg leaf mol^{-1} m^{-2} d^{-1}) of stockpiled or clipped (5-cm or 10-cm residue height) Jesup+, Jesup−, and MaxQ™ as a function of site. Vertical bars indicate standard error of the mean.

ity in LDMC between sites (eigenvalue, 0.0725). These observations concur with previously reported microclimate characteristics of the sites represented here (Belesky, 2005).

Radiation productivity offers a way to compare plant response to resource differences by integrating site environmental and plant physiological attributes, and provides some idea of how efficiently a management system is functioning. The RP was influenced by interaction of host–endophyte association, residue height and site (Table 1). Radiation productivity was greater for plants grown at SE than at NE or OP sites (Fig. 1) and concurs with radiation use efficiency trends reported for orchardgrass and tall fescue (Feldhake and Belesky, 2009). The RP of host–endophyte associations were similar for OP and NE, but were less at the SE site when stockpiled. Stockpiled J− at SE had a much lower RP than did similarly managed J+ or MaxQ™, suggesting some fundamental difference in metabolism that might be associated with infection status.

Endophyte-infected plants that were stockpiled, regardless of whether the endophyte was native or modified, could have a competitive advantage over non-infected plants when grown in partially shaded conditions and support observations reported by Clay and Leuchtmann (1989) for woodland grasses. Stockpiled plants were less likely to become carbon-depleted throughout the growing season because no intermittent clipping and recover cycles were imposed. When plants were clipped, there was less variation in RP at the 10 compared to the 5 cm residue height (based on greater R^2 for each host–endophyte association at 10 cm compared to 5 cm), but the response trends were similar.

Plant response to site represented by ET_o is shown clearly in LDMC, which when presented as a function of ET_o produced a strong linear response (Fig. 2). Leaf dry matter content is a direct product of light capture and conversion. Season-long LDMC means were significantly influenced by site and were greatest in plants produced at OP, and declined significantly (Table 1) as light availability declined (Fig. 2). The response to site occurred for stockpiled and clipped plants. There were minimal differences in LDMC as a function of host–endophyte associations. Clipping plants to a 10-cm residue resulted in greater LDMC compared to plants clipped to a 5-cm residue, although the greatest LDMC was obtained by stockpiling herbage (Fig. 2). High LDMC might suggest slow-growing plants such as those in stockpiled canopies (Gross et al., 2007).

Kannadan and Rudgers (2008) noted that a native plant (grove bluegrass; *Poa alsodes*) infected with a *Neotyphodium* endophyte had greater LDMC compared to non-infected plants when water stressed, and proposed that the response was consistent with mechanisms enabling plants to tolerate water deficit. Nutrient availability also could influence LDMC (Gross et al., 2007). In our experiment, N, P, and K were provided at each site to minimize

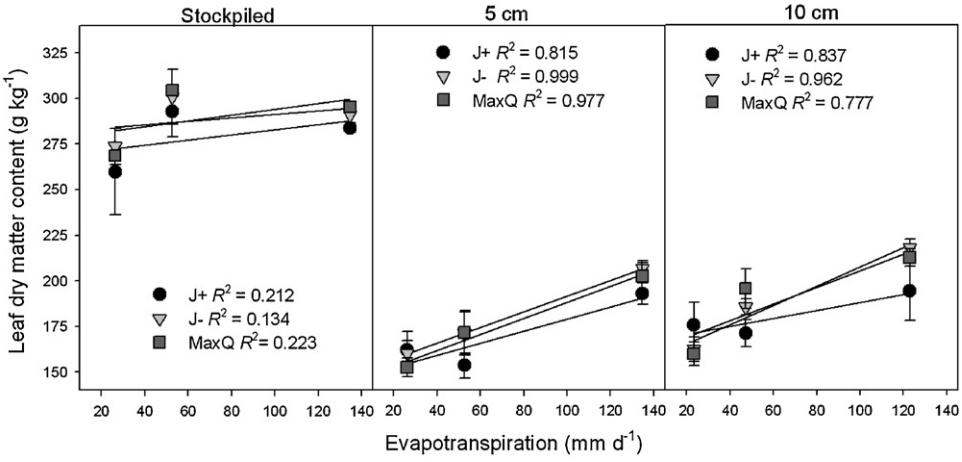

Fig. 2. Leaf dry matter content (g kg^{-1}) of clipped (5-cm or 10-cm residue height) Jesup+, Jesup−, and MaxQ™ as a function of potential evapotranspiration (ET_o, mm d^{-1}) representing the sites (OP, full sunlight; NE; about 60% of full sunlight; SE, about 20% of full sunlight). Vertical bars indicate standard error of the mean.

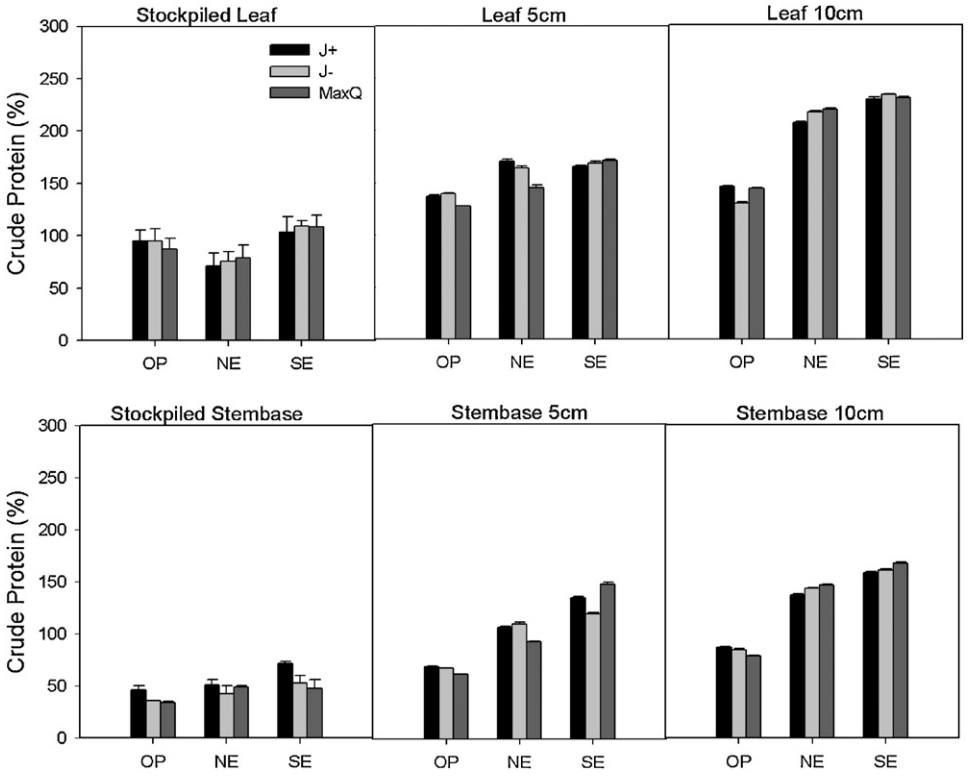

Fig. 3. Crude protein (CP, g kg^{-1}) of stockpiled or clipped (5-cm or 10-cm residue height) Jesup+, Jesup−, and MaxQ^{TM} as a function of site (OP, full sunlight; NE; about 60% of full sunlight; SE, about 20% of full sunlight). Vertical bars indicate standard error of the mean.

differences that might be caused by nutrient supply differences attributable to site.

3.2. Crude protein, total non-structural carbohydrate and herbage energy value

Despite uniform applications of N, herbage N concentration, expressed as CP, varied with site (Table 1). Crude protein concentrations in leaves of clipped plants were influenced by the interaction of site and residual sward height (Table 1). Plants clipped to 5 cm were similar regardless of site, but when clipped to the 10 cm residue height, plants had 30–40% more CP when growing at NE and SE than at OP (Fig. 3). The CP concentration of leaves did not differ between the SE and NE sites, but stembase CP did, with an increase in CP as light availability decreased. The CP of herbage from stockpiled plants was influenced by site, and was significantly less than that from plants clipped to either 5 cm or 10 cm. Nutritive value, based on CP only, was slightly greater for shade-grown (*i.e.*, NE and SE) compared to plants growing at OP. High leaf N concentrations, like high LDMC, were associated with relatively slow growth rates in some situations (Wardle et al., 1998). So in this instance, plants growing at OP while not light-limited, were likely to encounter drought stress, and plants growing at NE and SE were experiencing competition for water and light that were reflected in low LDMC and high N concentrations. The simultaneous stresses associated with light and water availability would influence photosynthate production and allocation.

Total non-structural carbohydrates accumulate in the stembases of cool-season grasses when photosynthate production exceeds growth demand. Concentrations of TNC exceeded 400 g kg^{-1} TNC in host–endophyte associations stockpiled for an entire growing season, regardless of site (Fig. 4). Site and residue height interacted to influence stembase TNC of clipped plants, but no clear trends occurred. Leaves of stockpiled plants had the most TNC at NE (220 g kg^{-1}) and the least (about 90 g kg^{-1}) at SE, with OP plants intermediate at about 150 g kg^{-1}. Concentrations of TNC in clipped plant stembases were about one-fifth of that occurring for stockpiled stembases. Interaction of residue height and site influenced TNC in leaves of clipped plants but differences were slight (Fig. 4). Leaf TNC of clipped plants was generally less than 50 g kg^{-1} and did not differ with respect to host–endophyte association (Table 1).

In terms of practical applications, accumulated N is of little value to ruminant livestock if insufficient energy is available for rumen microbial metabolism. Once N is assimilated by the plant, the process of conversion to structure or storage is linked closely with tissue N and current photosynthate supply. Nutritive value expressed as energy relative to CP reflects total digestible nutrients and in turn metabolizable energy and is an acceptable indicator of quality forage for grazing livestock (Neel et al., 2008).

When expressing TDN relative to CP (Neel et al., 2008), we found that stockpiled plants grown at OP and NE were acceptable for efficient CP use by the ruminant, while herbage from SE had insufficient energy (TNC), which could lead to inefficient N use (Fig. 5). There were statistically significant differences attributable to the interaction of site and host–endophyte association when plants were clipped to either the 5 cm or 10 cm residue (Table 1), but the differences were small (Fig. 5) and probably would not translate into practical differences. There were differences associated with site (Table 1) when plants were stockpiled (Fig. 5). All treatment combinations for clipped plants appeared to be energy deficient, reflecting the effects of repeated defoliation and site conditions on readily available carbohydrates. The decrease in TDN:CP in uncut plants with increasing shade corresponds with early discoveries concerning the nutritive value of shade-grown herbage (Deinum et al., 1968) and is consistent with results obtained for orchardgrass (Belesky et al., 2006). Clipped herbage might be substandard from the standpoint of N-use efficiency by the rumen biota, but satisfactory in terms of total energy or protein concentrations found

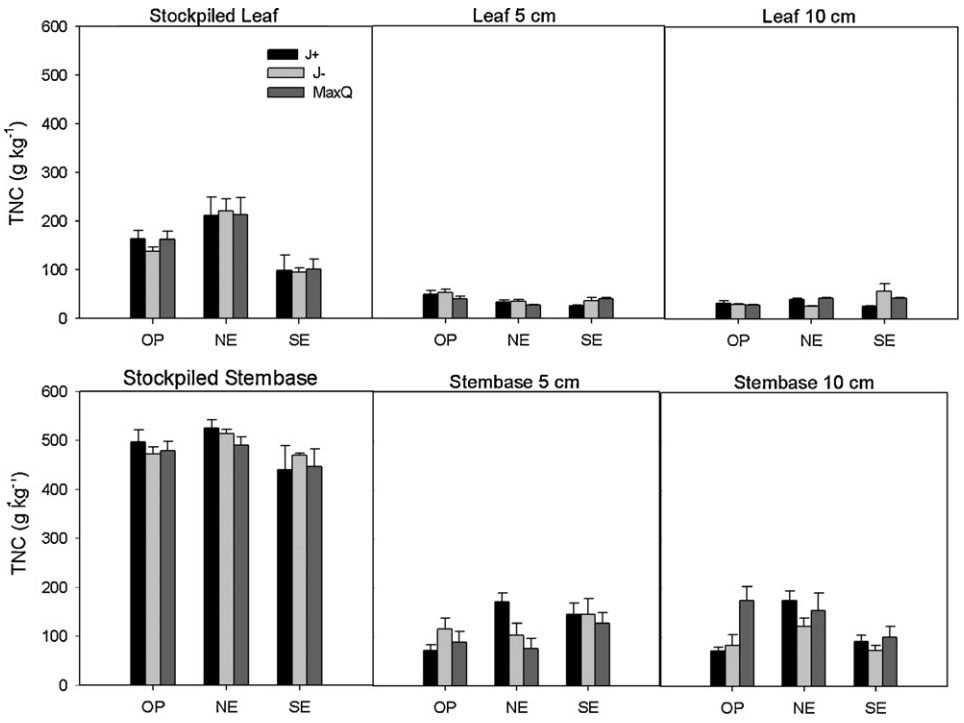

Fig. 4. Total non-structural carbohydrates (TNC, g kg^{-1}) of stockpiled or clipped (5-cm or 10-cm residue height) Jesup+, Jesup−, and MaxQ^{TM} as a function of site (OP, full sunlight; NE; about 60% of full sunlight; SE, about 20% of full sunlight). Vertical bars indicate standard error of the mean.

in the available herbage. In fact, Neel (personal communication) found liveweight gains for growing lambs (*Ovis* spp.) to be similar for traditional and silvopasture, but blood urea nitrogen data suggests inefficient N use by the grazer (Neel and Belesky, 2006).

3.3. Secondary metabolites; nitrates, loline and ergopeptide alkaloids, phenolics

Nitrate concentrations (g 100 g^{-1}) were least for herbage grown at OP and greater in shade-grown herbage, regardless of endophyte association (OP, 0.061 ± 0.03; NE, 0.89 ± 0.11; or SE, 1.60 ± 0.16). Herbage nitrate concentrations were negligible and did not differ among host–endophyte associations at the OP or the NE. Herbage nitrate concentrations were least in J+ and greatest in MaxQ^{TM}, with J− intermediate, at the SE site ($P \leq 0.10$). This supports previous observations that N assimilation was more efficient in endophyte-infected plants (Arachevaleta et al., 1989), and now recognizing that differences arising from endophyte strain may occur (Rasmussen et al., 2008). Likewise, the relatively lesser nitrate concentrations in J+ agrees with decreased nitrate concentrations in endophyte-infected plants observed by Rasmussen et al. (2008).

We know that repeated clipping can influence alkaloid concentrations in host–endophyte associations (Malinowski and Belesky, 2006). The creation of novel host–endophyte associations devoid of ergoalkaloids was a significant technological advance to help mitigate the occurrence and impact of fescue toxicosis in grazing livestock (Bouton and Easton, 2005). Understanding of how tall fescue hosting novel endophytes responds to management and environment is still emerging. Weather conditions also influence alkaloid concentrations and content, most likely through influences on the patterns and synchronicity of growth and metabolism of the symbionts.

Loline alkaloid concentrations differed as a function of host–endophyte association (Table 2). Concentrations were great-

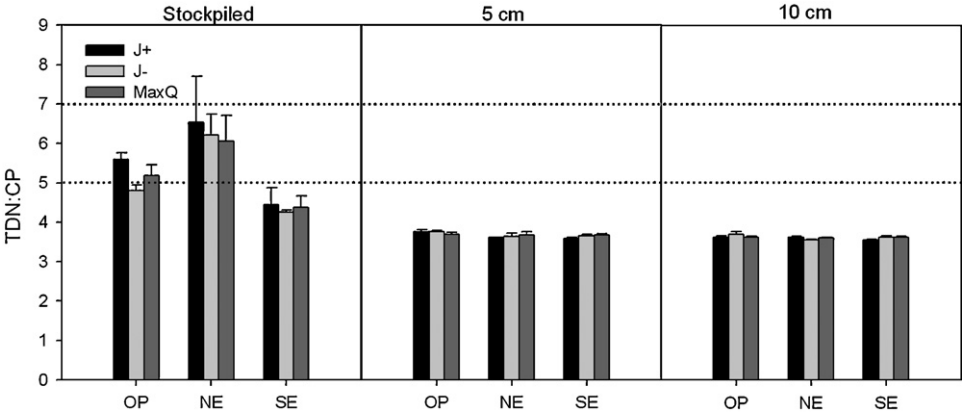

Fig. 5. The quotient of total digestible nutrients to crude protein (TDN:CP) of stockpiled or clipped (5-cm or 10-cm residue height) Jesup+, Jesup−, and MaxQ^{TM} as a function of site (OP, full sunlight; NE; about 60% of full sunlight; SE, about 20% of full sunlight). Dashed lines represents TDN:CP quotient range for optimal rumen nutrient conversion efficiency. Vertical bars indicate standard error of the mean.

Table 2
Analysis of variance for loline and ergoalkaloids in leaves, and total phenolics (μg 100 g^{-1}) in leaves and stembases, of Jesup+, Jesup−, and MaxQ™ tall fescue associations (FA) stockpiled or clipped to a 5-cm or 10-cm residue height (RH) as a function of site (S) including OP, full sunlight; NE; about 60% of full sunlight; SE, about 20% of full sunlight, for data representing repeated measurements of three replicates during the growing season.

Clipped	Lolines (96)[a]		Ergoalkaloids (90)[b]		Phenolics (96)			
					Leaf		Stembase	
	F	*P* > *F*	*F*	*P* > *F*	*F*	*P* > *F*	*F*	*P* > *F*
FA	10.09	**	–	–	2.11	ns	2.11	ns
S	3.91	ns	0.10	ns	68.11	***	68.11	***
RH	0.21	ns	3.49	ns	121.00	***	121.00	***
FA*S	1.17	ns	–	–	46.94	***	46.94	***
FA*RH	2.13	ns	–	–	27.00	***	27.00	***
S*RH	0.71	ns	0.84	ns	1.00	ns	1.00	ns
FA*S*RH	0.37	ns	–	–	5.50	**	5.50	**
Stockpiled								
FA	2.67	ns	–	–	–	–		
S	0.98	ns	4.79	*P* = 0.069	–	–		
FA*S	4.06	*	–	–	–	–		

ns, not significant.
[a] d.f., d.f. = 9 for stockpiled ergoalkaloid and 27 for stockpiled loline analysis.
[b] Statistical analysis for J+ only since ergoalkaloids were not detected in J− or MaxQ™.
* *P* < 0.05.
** *P* < 0.01.
*** *P* < 0.001.

est in J+, somewhat less in MaxQ™ and absent from J− plants subjected to simultaneous stresses associated with shading and defoliation (Fig. 6). Mixed model statistical analysis did not reveal any significant effects on loline concentrations other than fescue association (Table 2). We attribute this to the limited number of and variation associated with sample dates and the variation that occurs when environment and management interact over time. Based on means and standard errors of the mean, loline concentrations were greater in J+ plants at NE relative to OP when clipped to either the 5 cm or 10 cm residue height. Loline concentrations at SE were less than those obtained at NE, and similar to OP. When J+ plants were not cut (stockpiled), loline concentrations decreased with decreasing light. This could represent a dilution effect caused by relatively substantial accumulation of herbage mass coupled with unchanging alkaloid concentrations. Loline alkaloid concentrations in MaxQ™ increased as the amount of available light decreased, regardless of residual clipping height. The tendency for loline concentrations to increase in clipped endophyte-infected plants as light availability decreases might be related to N accumulation in shade-grown plants.

Ergoalkaloids differed as a function of host–endophyte association and were produced by J+ but not J− or MaxQ™ plants (Fig. 6). Ergoalkaloid concentrations decreased slightly (*P* = 0.069; d.f. = 9) with decreasing light availability in canopies that were not cut (Table 2). The decline could be related to disrupted or restricted photosynthate supply that occurs with the simultaneous stresses of defoliation and shade (Belesky and Hill, 1997). Ergoalkaloid concentrations in J+ plants were not influenced by site or residual clipping

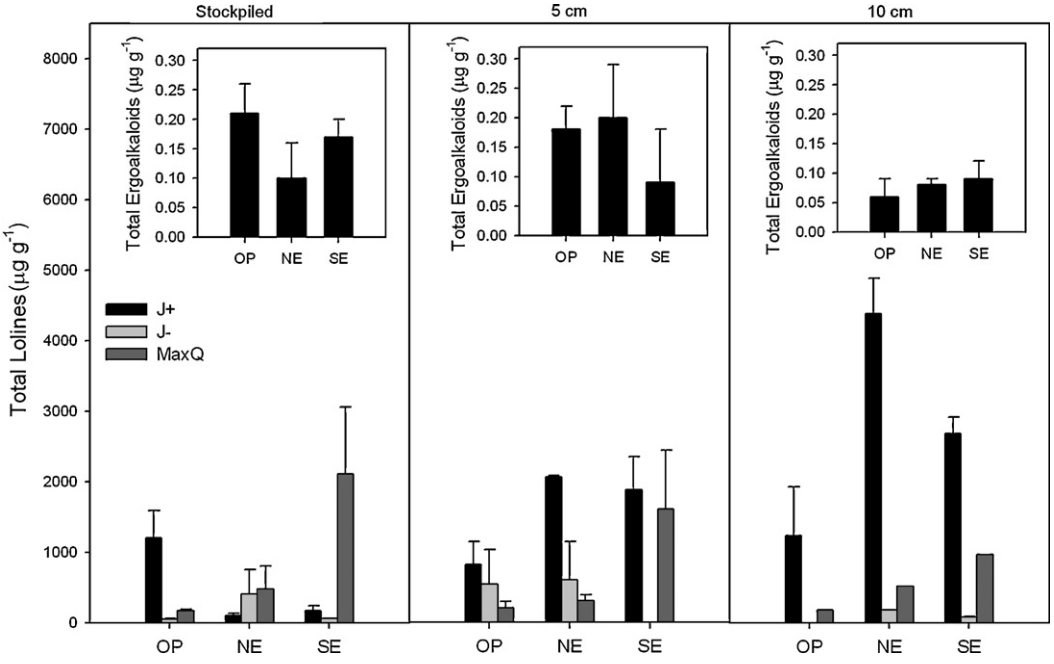

Fig. 6. Total lolines and ergoalkaloids (μg 100 g^{-1}) in harvested leaves of Jesup+, Jesup−, and MaxQ™ clipped to a 5-cm or 10-cm residue height as a function of site (OP, full sunlight; NE; about 60% of full sunlight; SE, about 20% of full sunlight). Vertical bars indicate standard error of the mean.

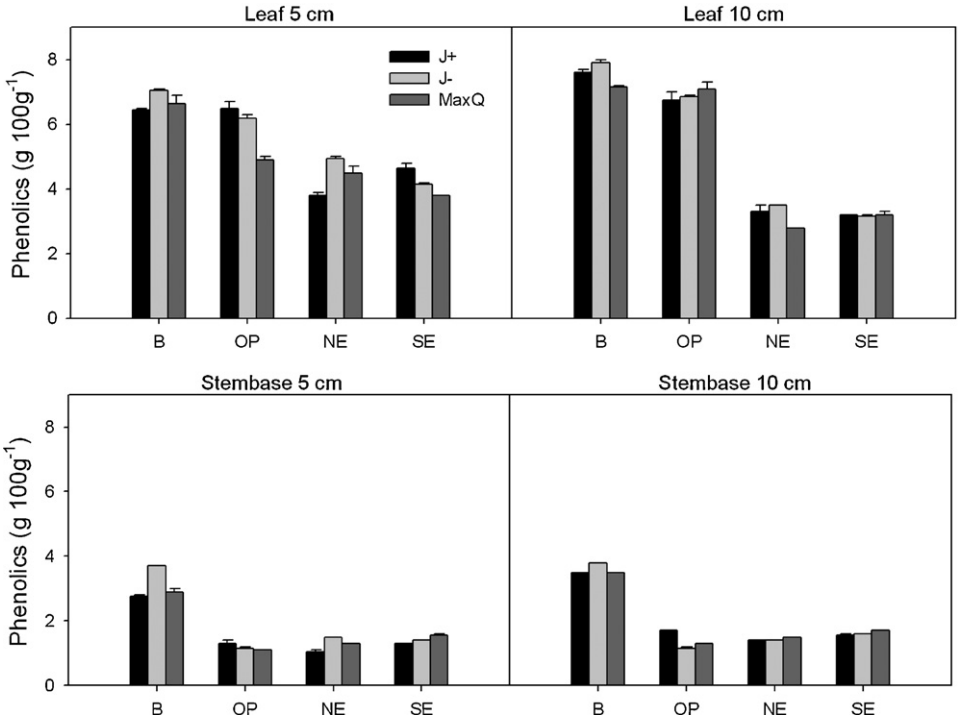

Fig. 7. Total phenolics (μg 100 g^{-1}) in harvested leaves of Jesup+, Jesup−, and MaxQ^{TM} clipped to a 5-cm or 10-cm residue height as a function of site (B, baseline; OP, full sunlight; NE; about 60% of full sunlight; SE, about 20% of full sunlight). Vertical bars indicate standard error of the mean.

height (Table 2). Absolute concentrations were similar in stockpiled and 5-cm residual height plants and were generally greater in stockpiled and 5-cm residue plants than in plants clipped to 10 cm. Some of the variation in ergoalkaloid concentration could arise from the amount of stembase occurring in the herbage sample since endophyte is localized in that region of the plant.

High phenolics concentrations in plants were linked with high light, high temperature, and restricted soil water availability (Burns, 1966). Concentrations of undifferentiated phenolics in leaf blades of tall fescue decreased as available light decreased (Fig. 7). Concentrations decreased about 27% when comparing OP to SE plants clipped to a 5-cm residue. Concentrations decreased about 50% for the same comparison when plants were clipped to a 10-cm residue. Concentrations were greater in J+ than in J− or MaxQ^{TM} when grown at OP. Plant tissues representing juvenile plants (baseline) had the greatest concentrations of phenolics prior to placement in the respective sites and support observations of high concentrations of phenolics accumulating in immature plant tissues and structures (reviewed in Herms and Mattson, 1992). Leaf tissue generated at OP had concentrations similar to that of the baseline plants, whereas stembase tissue concentrations were about half of that occurring in plants growing at the other sites. Stembase phenolic concentrations did not differ as a function of host–endophyte association and increased slightly with decreasing light availability, especially in MaxQ^{TM}. Accumulation of phenolics in leaf blades of plants growing at OP could provide photo-oxidation stress protection, and be a means to deter herbivores or contribute to meristem protection when accumulating in stembases of J− and MaxQ^{TM} under shaded conditions (Close and McArthur, 2002). The decline in phenolic constituents and accumulation of N in shade-grown plants may increase susceptibility to herbivory and eventual stand decline since current photosynthate would very likely be insufficient for vigorous regrowth after a defoliation event. However, changes in specific secondary constituents are more likely to represent responses to light quality than simple undifferentiated analyses (*e.g.*, total phenolics) (Roberts and Paul, 2006).

4. Conclusions

Radiation productivity and LDMC of tall fescue were comparable in partially shaded (40% light attenuation) and open (full sunlight) sites, suggesting that tall fescue could be grown in modest shade associated with certain silvopastoral practices. The RP and LDMC varied less with respect to site conditions when plants were infected with a native endophyte compared to non-infected plants or plants hosting a novel, non-ergogenic endophyte. The trend suggests that native endophyte contributed to resilience of the host, including somewhat more efficient N use reflected in lesser nitrate concentrations. Patterns of TNC accumulation reflected the interaction of site and clipping management on the acquisition and utilization of photosynthate. When tall fescue was stockpiled in a partially shaded site, such as the NE site with an average 40% light attenuation, the resulting dry matter production and nutritive value would meet the needs of grazing livestock. However, when clipped repeatedly, regardless of host–endophyte association or residual plant height, productivity and nutritive value might not be sufficient to sustain grazer productivity. The TNC patterns might be worth considering with respect to other ecophysiological indices that integrate plant response to environmental features over time. Ergoalkaloid concentrations in the native endophyte–host association were greater when plants grew in full sunlight and seemed to be influenced by readily available photosynthate. The concentration of loline alkaloids, irrespective of host–endophyte association, tended to increase in leaves with decreasing light availability and could be related to the relatively greater N concentrations and non-structural carbohydrate depletion occurring in shade-grown leaves. Phenolics decreased in leaves, but increased in stembases as light availability decreased. The relatively greater concentration of phenolics in light-grown compared to shade-grown leaves supports the hypothesis that phenolics occur to help protect the plant from photo-damage (Close and McArthur, 2002). The combination of increased loline alkaloids in leaves and phenolics in stembases, suggests that shade-grown tall fescue might have some competitive

advantage based on the known anti-herbivory attributes of loline alkaloids and phenolic compounds.

Acknowledgements

We thank C.M. Feldhake, USDA-ARS, AFSRC for creating the forest clearing research site, and J.A. Rudgers, Rice University, for valuable suggestions to improve the draft manuscript. We also thank Mr. M.L. Huffman, Mr. J.M. Peele and Mr. C.E. Ellison for excellent technical assistance; Mr. Donald Wood, University of Georgia and Dr. J.H. Bouton, The Samuel Roberts Noble Foundation for providing seeds; and anonymous reviewers who helped improve the presentation of our findings.

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